Invitro Anti-Inflammatory Activity and Qualitative Bio-Chemical Analysis of Polyherbal Siddha Formulation - Vaathathukku Chooranam

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Abstract: Siddha system was propounded by the siddhars and which a vast and unique system which defines Health as a prefect state of physical, psychosocial, social and spiritual wellbeing of an individual. In siddha system, siddhars classified diseases into 4448. Yagiswam translated 80 types of vaatha diseases in his text. Yugi padaitha chinthamani. In which “Uthiravaatha Sronitham” is one among them and the signs and symptoms of this disease is correlated with Rheumatoid arthritis. Siddha system effectively treats chronic diseases like rheumatoid arthritis, diabetes etc. Traditional siddha medicines offer a wide range of Anti-inflammatory drugs which are polyherbal in nature. This research paper deals with Anti-inflammatory activity and bio-chemical analysis of Vaathathukku Chooranam documented in classical siddha text Agathiyar maruthuvam indicated for Vaatham (Arthritis). This study confirms the Vaathathukku Chooranam possess Anti-inflammatory activity.

Keywords: Rheumatoid arthritis, Uthiravaatha sronitham, Vaathathukku Chooranam, Anti-inflammatory activity

1. Introduction

Rheumatoid arthritis is a chronic, symmetric, inflammatory peripheral polyarthritis of unknown aetiology. The prevalence of Rheumatoid arthritis is approximately 0.8 - 1.0% in Europe and Indian subcontinent. The incidence of Rheumatoid arthritis is relatively three times higher in females as compared to the males. It affects approximately 0.5% of the adult population worldwide and occurs 20-50 cases per 1,00,000 annually, mainly in women after their 40’s. Rheumatoid arthritis is associated with a variety of extra articular co-morbidities, including cardiovascular disease, resulting in increased mortality in patients with Rheumatoid arthritis. The usage of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of painful musculoskeletal conditions often results in adverse effects such as skin rashes, gastric irritation etc. By the way, polyherbal medicines are potent, efficient, time-tested and devoid of severe side-effects. Hence this current study was carried out to prove the Anti-inflammatory activity of Vaathathukku Chooranam by invitro assays and bio-chemical analysis.

2. Materials and Methods

Drug Selection

The siddha formulation Vaathathukku Chooranam taken from the Agathiyar maruthuvam and it is indicated for Vaatham (Arthritis).

Ingredients of Vaathathukku Chooranam:

1) Seeragam (Cuminum cyminum)
2) Karunjeeragam (Nigella sativa)
3) Sathakuppai (Anethum graveolens)
4) Chukku (Zingiber officinalis)
5) Milagu (Piper nigrum)
6) Thippili (Piper longum)
7) Kodiveli (Plumbago indica)

Table 1: Information about the Ingredients of Vaathathukku Chooranam

<table>
<thead>
<tr>
<th>S. No</th>
<th>Common name / Familly</th>
<th>Botanical name / Family</th>
<th>Phytochemistry</th>
<th>Actions</th>
<th>Uses in siddha</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>Sathakuppai/Dill</td>
<td>Anethum graveolens</td>
<td>Carvone, Limonene, Cineole, α-</td>
<td>Anti-inflammatory</td>
<td>Vaatha disease, headache,</td>
</tr>
</tbody>
</table>

Volume 11 Issue 4, April 2022

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Paper ID: MR22412165339 DOI: 10.21275/MR22412165339 1024
4. Chukku/ Dried ginger: Zingiber officinalis / Zingiberaceae
   - Constituents: Zingiberene, Curcumene, Zingerone, Gingerol, D-camphor, Zingerberols, Farnensene

5. Milagu/Black pepper: Piper nigrum/ Piperaceae
   - Constituents: Phenolic amino acids, Alpha tocopherol, Feruiperine, Butylated hydroxytoluene, Butylated hydroxyanisole
   - Assay Procedure: Anti-tumour, Anti-oxidant, Anti-bacterial, Anti-asthmatic, Anti-hypertensive, Anti-platelet aggregation

6. Thippili/ Long pepper: Piper longum/ Piperaceae
   - Constituents: Piperine, Piperttine, Piper longumine, Piper longumine, Piperideine, Asarining, Pellitorine
   - Assay Procedure: Anti-oxidant, Anti-cancer, Anti-ulcer, Anti-microbial, Anti-coagulant

7. Kodiveli/ Indian leadwort: Plumbago indicol / Plumbaginaceae
   - Constituents: Plumbagin, Sitosterol, Stigmasterol, β-Ayrin, α-sitosterol, 4-hapthoguineone, 5,6 dihydroxy-2-methyl-1
   - Assay Procedure: Anti-cancer, Anti-bacterial, Anti-oxidant, Anti-microbial, Anti-inflammatoryy, Anti-convulsant

Collection of Raw Drugs:
The drugs are purchased from ASN herbal drug shop, Melapalayam, Tirunelveli.

Authentication of Raw Drugs:
The identification of polyherbal drugs are authenticated by faculties of Department of Gunapadam, Government siddha medical college and Hospital, Palayamkottai.

Method of Purification:
All the raw drugs are purified as per the methods mentioned in Siddha literature.

Method of Drug Preparation:
Vaathathukku Chooranam was prepared according to the procedure mentioned in Siddha classical text Agathiyar maruthuvam.

Figure 1: Vaathathukku Chooranam

Anti-Inflammatory Activity:

Albumin Denaturation Assay Procedure:
In-vitro anti-inflammatory activity VAC as studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample chloroform extract of VAC at varying concentration ranges from 100 to 500 µg/ml along with standard Diclofenac sodium at the concentration of100 µg/ml of final volume. pH was adjusted by using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity developed was measured spectrophotometrically at 660 nm, for control distilled water was used instead of test sample while product control tests lacked bovine serum albumin. The experiment was performed in triplicate.

The Percentage protection from denaturation is calculated by using the formula

\[
\left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100.
\]

Biochemical Analysis:
Preparation of the extract:
5 grams of the drug was weighed accurately and placed in a 250 ml clean beaker, then 50 ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100 ml volumetric flask and then it is made to 100 ml with distilled water. This fluid is taken for analysis.

3. Results and Discussion

Anti-Inflammatory Activity:
Results are expressed as Mean ± SD. The difference between experimental groups was compared by One-Way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test.
Table 2: Invitro Anti-inflammatory activity of Vaathathukku Chooranam

<table>
<thead>
<tr>
<th>Concentration in µg/ml</th>
<th>Percentage Inhibition of Protein Denaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAC 100</td>
<td>8.674 ± 1.376</td>
</tr>
<tr>
<td>VAC 200</td>
<td>23.64 ± 2.248</td>
</tr>
<tr>
<td>VAC 300</td>
<td>31.55 ± 2.84</td>
</tr>
<tr>
<td>VAC 400</td>
<td>53.01 ± 2.055</td>
</tr>
<tr>
<td>VAC 500</td>
<td>64.47 ± 1.781</td>
</tr>
<tr>
<td>Diclofenac sodium (100 µg)</td>
<td>95.39 ± 1.226</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD. N=3

Percentage Inhibition of Protein Denaturation by VAC and Standard

The result obtained from the present clearly indicates that the test drug VAC was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 64.47 ± 1.781 % was observed at 500µg/ml when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 95.39 ± 1.226 at the concentration of 100 µg/ml.

Vaathathukku Chooranam is the combination of most efficacious ingredients. While evaluating the ingredients seeragam, sathakuppai, chukku, kodiveli and are all exhibits anti-inflammatory property. Along with this, seeragam possess Anti-coagulant activity and sathakuppai have a Analgesic activity which aids for subsiding inflammatory signs.

VAC*-Vaathathukku Chooranam

Figure 2: Mean Percentage inhibition of Albumin protein denaturation by Siddha formulation VAC

Table 3: Test Ffor Acidic Radicals

<table>
<thead>
<tr>
<th>S. No</th>
<th>Procedure</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Test for calcium:</td>
<td>To 2 ml of the above prepared extract taken in a clean test tube. To this add 2 ml of 4% ammonium oxalate solution.</td>
<td>White precipitate is formed</td>
</tr>
<tr>
<td>2.</td>
<td>Test for sulphate:</td>
<td>To 2ml of the extract is added to 5% barium chloride solution.</td>
<td>White precipitate is formed</td>
</tr>
<tr>
<td>3.</td>
<td>Test for chloride:</td>
<td>The extract is treated with silver nitrate solution.</td>
<td>White precipitate is formed</td>
</tr>
<tr>
<td>4.</td>
<td>Test for carbonate:</td>
<td>The substance is treated with concentrated HCL.</td>
<td>No brisk effervescence is formed</td>
</tr>
<tr>
<td>5.</td>
<td>Test for Starch:</td>
<td>The extract is added with weak iodine solution.</td>
<td>Blue colour is formed</td>
</tr>
<tr>
<td>6.</td>
<td>Test for ferric iron:</td>
<td>The extract is acidified with glacial acetic acid and potassium ferro cyanide.</td>
<td>No blue colour is formed</td>
</tr>
<tr>
<td>7.</td>
<td>Test for ferrous iron:</td>
<td>The extract is treated with concentrated nitric acid ammonium thiocyanate solution.</td>
<td>Blue colour is formed</td>
</tr>
<tr>
<td>8.</td>
<td>Test for phosphate:</td>
<td>The extract is treated with ammonium molybdate and concentrated nitric acid.</td>
<td>Yellow precipitate is formed</td>
</tr>
<tr>
<td>9.</td>
<td>Test for albumin:</td>
<td>The extract is treated with esbach’s reagent.</td>
<td>No yellow precipitate is formed</td>
</tr>
<tr>
<td>10.</td>
<td>Test for tannic acid:</td>
<td>The extract is treated with ferric chloride.</td>
<td>No blueblack precipitate is formed</td>
</tr>
<tr>
<td>11.</td>
<td>Test for unsaturation:</td>
<td>Potassium permanganate solution is added to the extract.</td>
<td>It gets decolourised</td>
</tr>
<tr>
<td>12.</td>
<td>Test for the reducing sugar:</td>
<td>To 5 ml of benedict’s qualitative solution is taken in a</td>
<td>Colour changes occurs</td>
</tr>
</tbody>
</table>
test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes.

<table>
<thead>
<tr>
<th>13.</th>
<th><strong>Test for amino acid:</strong></th>
<th>Violet colour is formed</th>
<th>Presence of amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayed over the same and dried it well.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>14.</th>
<th><strong>Test for zinc:</strong></th>
<th>No white precipitate is formed</th>
<th>Absence of zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The extract is treated with Potassium Ferro cyanide.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The qualitative bio chemical analysis of Vaathathukku Chooranam reveals the presence of Calcium, Sulphate, Chloride, Starch, Ferrous iron, Phosphate, Unsaturation compound, Reducing sugar, Amino acid.

This study reveals the presence of Calcium which is very essential constituent of bones, Muscle contraction and nerve transmission and phosphate also have a constituent of bone and teeth. The presence of chloride which is involved in the regulation of acid-base equilibrium. It also ensures that presence of ferrous iron, that helps to increase in the formation of new blood cells which always affected in inflammatory conditions.

4. **Conclusion**

From this study, we can conclude that the polyherbal formulation of Vaathathukku Chooranam possess significant anti-inflammatory property and it contains phytochemicals, so it is the most promising drug for Rheumatoid arthritis. In future in-vivo study will be done on Vaathathukku Chooranam for further extensive research.

5. **Acknowledgement**

I wish to express my sincere thanks to Dr. A. Balamurugan, Lecturer Grade -II, Department of Noi Naadal, GSMC, Palayamkottai, for the valuable support.

**References**


